Supplemental Material

DipM, a new factor required for peptidoglycan remodeling during cell division in *Caulobacter crescentus*

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SUPPLEMENTAL METHODS

Protein purification

To overproduce His-tagged derivatives of DipM or MalE, *E. coli* strain Rosetta(DE3)/pLysS (Invitrogen) was transformed with plasmids pAM072, pAM091, pAM092 or pAM142, respectively. The resulting strains were grown to an OD_{600} of 1.0 in SB medium at 37°C. IPTG (isopropyl-β-D-thiogalactoside) was added to a final concentration of 0.5 mM and incubation was continued for 3 h. Cells were harvested by centrifugation for 10 min at 7500 x g and 4°C, washed in 200 ml buffer 1 (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, adjusted to pH 8.0 with NaOH) and frozen in liquid nitrogen. Proteins were purified by nickel affinity chromatography followed by ion exchange chromatography ('MAS'-$DipM_{AA26-609}$) or size exclusion chromatography.

Cells were resuspended in 2 ml/g cell extract buffer 1 containing 100 µg/ml phenylmethylsulfonyl fluoride (PMSF) and 10 µg/ml DNase I and lysed by four passages through a French press at 16000 psi. The suspension was centrifuged for 30 min at 10.000 x g, 4°C. The supernatant was loaded on a Ni-NTA agarose column (Qiagen) equilibrated with 40 ml buffer 2 (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, adjusted to pH 8.0 with NaOH). The column was washed with 100 ml of buffer 2, and protein was eluted with 30 ml imidazole gradient from 20 to 250 mM imidazole followed by 30 ml buffer 3 (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, adjusted to pH 8.0 with NaOH), collecting 2 ml fractions. Fractions containing the protein of interest were pooled. For ion exchange chromatography, the sample was dialyzed against buffer 4 (50 mM NaH_2PO_4, pH 7.0, 5 mM $MgCl_2$, 0.1 mM EDTA) containing 50 mM NaCl. The solution was centrifuged 20 min at 13.000 x g (4°C) and loaded on an SP Sepharose Fast Flow column (GE Healthcare) equilibrated with buffer 4 containing 50 mM NaCl. The column was washed with 60 ml of the same buffer, and protein was eluted with 200 ml of a linear NaCl gradient (50-500 mM NaCl in buffer B5), collecting 5 ml fractions. Fractions containing the protein of interest were pooled, dialyzed against 50 mM NaH_2PO_4, pH 7.4, adjusted to 10% glycerol, aliquoted, snap frozen in liquid nitrogen, and stored at -80°C. For size exclusion chromatography, the sample was dialyzed against buffer 5 (50 mM NaH_2PO_4, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$). The sample was then applied to a Superdex 75 GL10/300 column (GE Healthcare) equilibrated with buffer 5, and protein was eluted with 25 ml buffer 5, collecting 2.5 ml fractions. Fractions containing the protein of interest were pooled, dialyzed against 50 mM NaH_2PO_4, pH 7.4, adjusted to 10% glycerol, aliquoted, snap frozen in liquid nitrogen, and stored at -80°C.

Co-immunoprecipitation analysis

C. crescentus strains CB15N (WT), MT46 (*egfp-ftsN*), and AM285 (*dipM-strepII*) were grown in M2G medium to an OD_{600} of 0.5. Formaldehyde was added to a final concentration of 0.6%, and the culture was incubated for 20 min at 37°C. The reaction was stopped by addition of glycine to final concentration of 125 mM and incubation for 5 min at room temperature. Cells were harvested by centrifugation at 7000 rpm, 4°C for 10 min, washed three times in 50 mM NaH_2PO_4 (pH 7.4), 5 mM $MgCl_2$, and frozen at −80°C. For analysis, cells were thawed on ice, washed with Co-IP buffer 1 (100 mM sodium/ potassium phosphate buffer, pH 7.8, 140 mM NaCl, 5 mM EDTA), and resuspended in 2.5 ml Co-IP buffer 1 per gram wet cell pellet. The suspension was supplemented with 10 mM $MgCl_2$, 10 mg/ml lysozyme, 10 U/ml DNaseI and 100 µg/ml PMSF and incubated at 4°C for 30 min. Cell were lysed by sonication, and Triton X-100 was added to a final concentration of 0.5%. After incubation for 1 h at 4°C, the solution was centrifuged for 15 min at 10,000 g (4 °C), and the protein concentration in the supernatant was adjusted to 40 mg/ml with Co-IP buffer 1. To precipitate the DipM-StrepII fusion, 80 µl Strep-Tactin magnetic beads (Qiagen) were washed three times with Co-IP buffer 2 (50 mM Tris/HCl, pH 7.5, 140 mM NaCl, 0.05% Tween-20) and then equilibrated with Co-IP buffer 1. 80 µl cell lysate and 80 µl Co-IP buffer 1 were added to the beads, and the suspension was incubated overnight at 4°C. After washing three times with Co-IP buffer 1, the beads were resuspended in 100 µl SDS sample buffer, heated to 95°C for 30 min, and sedimented by centrifugation. The supernatant was stored at -20°C and analyzed by immunoblotting. To immunoprecipitate eGFP-FtsN, 500 µl cell lysate was added to 50 µl anti-GFP antibody-coupled agarose slurry (MBL) and incubated overnight at 4°C. The matrix was washed three times with 500 µl cold Co-IP buffer 1, and bound protein was eluted as described above.

SUPPLEMENTAL TABLES

Table S1. Phenotypic characteristics of the Δ*dipM* mutant strain MT258.

	CB15N	**CB15N Δ*dipM*** (MT258)
total number of cells counted	83	117
average length (μm)	3.1 (± 1.0)	13.4 (± 9.8)
max. length (μm)	5.6	50.1
min. length (μm)	1.0	0.9
average number of chain links [a]	1.56 (± 0.5)	2.2 (± 1.2)
average length per chain link (μm)	2.1 (± 0.6)	7.9 (± 7.9)
percentage of cells containing branches	0.0	16.2
percentage of cells displaying chaining [b]	0.0	13.7

[a] chain links were defined as cells separated by visible constrictions
[b] chaining cells were defined as cells containing more than one constriction

Table S2. Phenotypic characteristics of strains expressing truncated *dipM* derivatives.

	AM222 *dipM*ΔAA123-166	**AM241** *dipM*ΔAA123-216	**AM234** *dipM*ΔAA123-340	**AM242** *dipM*ΔAA123-390	**SS187** *dipM*ΔAA123-291
total number of cells counted	50	50	50	50	50
average length (μm)	2.9 (± 0.9)	2.7 (± 1.0)	3.1 (± 0.9)	3.6 (± 0.6)	3.3 (± 1.1)
max. length (μm)	4.7	5.9	5.3	5.1	6.0
min. length (μm)	1.6	1.6	2.0	1.9	1.6
average number of chain links [a]	1.4 (± 0.5)	1.2 (± 0.4)	1.2 (± 0.4)	1.2 (± 0.4)	1.6 (± 0.5)
avg. length per chain link (μm)	2.1 (± 0.4)	2.2 (± 0.3)	2.6 (± 0.4)	3.1 (± 0.7)	2.1 (± 0.6)

[a] chain links were defined as cells separated by visible constrictions

Table S3. Strains used in this study.

Strain	**Genotype/description**	**Reference/source**
C. crescentus		
CB15N	synchronizable derivative of wild-type strain CB15 (NA1000)	Evinger & Agabian, 1977
CS606	CB15N Δ*bla*	West *et al.*, 2002
GB175	CB15N P*van*::P*van-popZ-eyfp*	Bowman *et al.*, 2008
LS4032	CB15N P*xyl*::P*xyl-egfp* + pEJ216	Judd *et al.*, 2005
NR1371	CB15N *tipN*::*tipN-egfp*	Huitema *et al.*, 2006
YB1585	CB15N *ftsZ*::P*xyl-ftsZ*	Wang *et al.*, 2001
AM52	CB15N Δ*vanA* Δ *ftsN* P*van*::P*van-ftsN*	Möll & Thanbichler, 2009
AM128	CB15N Δ*vanA* Δ *ftsN* P*van*::P*van-ftsN* P*xyl*::P*xyl-dipM-mCherry*	This work
AM192	CB15N P*xyl*::P*xyl-dipM*Δ(AA501-609)-*mCherry*	This work
AM199	CB15N P*xyl*::P*xyl-dipM*Δ(AA292-609)-*mCherry*	This work
AM200	CB15N P*xyl*::P*xyl-dipM*Δ(AA123-609)-*mCherry*	This work
AM205	CB15N Δ*dipM* P*xyl*::P*xyl-dipM-mCherry*	This work
AM206	CB15N P*xyl*::P*xyl-dipM-mCherry* P*van*::P*van-ftsZ-ecfp*	This work
AM214	CB15N *ftsZ*::P*xyl-ftsZ* P*van*::P*van-dipM-mCherry*	This work
AM216	CB15N *dipM-mCherry* P*xyl*::P*xyl-ftsK-ecfp*	This work
AM222	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-166)-*mCherry*	This work
AM225	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA501-609)-*mCherry*	This work
AM231	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA292-609))-*mCherry*	This work
AM232	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-609)-*mCherry*	This work
AM233	CB15N P*xyl*::P*xyl-dipM*Δ(AA123-340)-*mCherry*	This work
AM234	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-340)-*mCherry*	This work
AM240	CB15N P*xyl*::P*xyl-dipM*Δ(AA123-390)-*mCherry*	This work
AM241	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-216)-*mCherry*	This work
AM242	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-390)-*mCherry*	This work
AM263	CB15N Δ*dipM tipN*::*tipN-egfp*	This work
AM264	CB15N Δ*dipM* P*van*::P*van-popZ-eyfp*	This work
AM285	CB15N *dipM*::*dipM-strepII*	This work
AM296	CB15N Δ*dipM* P*xyl*::P*xyl-egfp* + pEJ216	This work
AM337	CB15N ΔCC1876 (*amiC*)	This work
AM342	CB15N Δ*bla* P*xyl*::P*xyl-dipM-bla*	This work
MT46	CB15N *egfp-ftsN*	Möll & Thanbichler, 2009
MT251	CB15N P*xyl*::P*xyl-dipM-mCherry*	This work
MT258	CB15N Δ*dipM*	This work
MT261	CB15N *dipM-mCherry*	This work
SS187	CB15N Δ*dipM* P*xyl*::P*xyl-dipM*Δ(AA123-291)-*mCherry*	This work
E. coli		
BTH101	*cya* BACTH reporter strain	Karimova *et al.*, 1998
Rosetta (DE3)/pLysS	protein overproduction strain	Novagen
TOP10	cloning strain	Invitrogen
XL1-Blue	cloning strain	Stratagene

Table S4. Plasmids used in this study.

Plasmid	Genotype/description	Reference/source
pBAD24-CB	Medium copy number plasmid carrying the arabinose inducible P_{BAD} promoter, Amp^R	Möll & Thanbichler, 2009
pBXMCS-2	Plasmid for overproduction of proteins in *C. crescentus*, Kan^R	Thanbichler *et al.*, 2007
pET28a(+)	Plasmid for protein overproduction in *E. coli,* Kan^R	Novagen
pEJ216	Replicating plasmid carrying *torA'-tdimer2* under control of P*xyl*, Cam^R	Judd *et al.*, 2005
pGB113	Integration plasmid carrying *popZ-eyfp* under control of P*van*, $Gent^R$	Bowman *et al.*, 2008
pKT25	Plasmid for constructing C-terminal fusions to T25, Kan^R	Euromedex
pMAL-c2e	Plasmid for constructing maltose-binding protein fusions, Amp^R	New England Biolabs
pMT217	Integration plasmid carrying P*xyl-ftsZ-eyfp*, Kan^R	Thanbichler & Shapiro, 2006
pNTPS138	*sacB*-containing suizide vector used for double homologous recombination, Kan^R	M.R.K. Alley, unpublished
pSPX47	Integration plasmid carrying *tipN-egfp*, Kan^R	Huitema *et al.*, 2006
pSTRC-2	Plasmid for constructing C-terminal fusions to Strep-tag II	Thanbichler *et al.*, 2007
pUT18C	Plasmid for constructing C-terminal fusions to T18, Amp^R	Euromedex
pXBlaMC-2	Plasmid for constructing C-terminal fusions to β-lactamase, Kan^R	S. Schliimpert, unpublished
pXCFPC-2	Integration plasmid for constructing C-terminal fusions to eCFP under the control of P*xyl*, Kan^R	Thanbichler *et al.*, 2007
pXCFPN-4	Integration plasmid for constructing N-terminal fusions to eCFP under the control of P*xyl*, $Gent^R$	Thanbichler *et al.*, 2007
pXCHYC-1	Integration plasmid for constructing C-terminal fusions to mCherry under the control of P*xyl*, $Spec/Str^R$	Thanbichler *et al.*, 2007
pXCHYC-2	Integration plasmid for constructing C-terminal fusions to mCherry under the control of P*xyl*, Kan^R	Thanbichler *et al.*, 2007
pVCFPC-6	Integration plasmid for constructing C-terminal fusions to eCFP under the control of P*van*, Cam^R	Thanbichler *et al.*, 2007
pVCHYC-1	Integration plasmid for constructing C-terminal fusions to mCherry under the control of P*van*, $Spec/Str^R$	Thanbichler *et al.*, 2007
pAM_T25pp	pKT25 carrying *malG*(AA1-77)	This work
pAM_T18pp	pUT18C carrying *malG*(AA1-77)	This work
pAM071	pXCHYC-2 carrying *dipM*Δ(AA501-609)	This work
pAM072	pET28a(+) carrying 'MAS'-*dipM*(AA26-609)	This work
pAM076	pXCHYC-2 carrying *dipM*Δ(AA292-609)	This work
pAM077	pXCHYC-2 carrying *dipM*Δ(AA123-609)	This work
pAM078	pXCHYC-2 carrying *dipM*Δ(AA123-166)	This work
pAM079	pXCHYC-2 carrying *dipM*Δ(AA123-340)	This work
pAM081	pXCHYC-2 carrying *dipM*Δ(AA123-216)	This work
pAM082	pXCHYC-2 carrying *dipM*Δ(AA123-390)	This work
pAM083	pXCHYC-1 carrying *dipM*	This work
pAM085	pVCHYC-1 carrying *dipM*	This work
pAM087	pXCFPC-2 carrying *ftsK*	This work
pAM091	pET28a(+) carrying 'MAS'-*dipM*(AA501-609)	This work
pAM092	pET28a(+) carrying 'MAS'-*dipM*(AA26-500)	This work
pAM100	pSTRC-2 carrying *dipM*	This work
pAM102	pUT18C carrying *malG*(AA 1-77)-CC1876(AA35-396)	This work
pAM103	pKT25 carrying *malG*(AA 1-77)-CC1876(AA35-396)	This work
pAM104	pUT18C carrying *malG*(AA1-77)-'MAS'-*dipM*(AA26-609)	This work
pAM105	pKT25 carrying *malG*(AA1-77)-'MAS'-*dipM*(AA26-609)	This work
pAM111	pKT25 carrying CC1546 (*pbp2*)	This work
pAM112	pUT18C carrying CC1546 (*pbp2*)	This work
pAM113	pKT25 carrying CC3232 (*tolR*)	This work
pAM114	pUT18C carrying CC3232 (*tolR*)	This work
pAM121	pKT25 carrying *malG*(AA1-77)- 'MAS'-*dipM*(AA501-609)	This work
pAM122	pUT18C carrying *malG*(AA1-77)- 'MAS'-*dipM*(AA501-609)	This work
pAM123	pNTPS138-based plasmid for constructing an in-frame deletion in CC1876 (*amiC*)	This work
pAM125	pKT25 carrying *malG*(AA1-77)- 'MAS'-*dipM*(AA1-500)	This work
pAM126	pUT18C carrying *malG*(AA1-77)- 'MAS'-*dipM*(AA1-500)	This work
pAM141	pXBlaMC-2 carrying *dipM*	This work
pAM142	pET28a(+) carrying *malE*	This work
pMT795	pXCHYC-2 carrying *dipM*	This work
pMT808	pBXMCS-2 carrying *dipM*	This work
pMT814	pNTPS138-based plasmid for constructing an in-frame deletion in *dipM*	This work
pMT816	pNTPS138-based plasmid for replacing the native *dipM* gene with a *dipM-mCherry* fusion	This work
pSS85	pBAD24-CB carrying *dipM-mCherry*	This work
pSS96	pUT18C carrying *C. crescentus ftsN* (CC2007)	This work
pSS102	pKT25 carrying *C. crescentus ftsN* (CC2007)	This work
pSS124	pKNT25 carrying *tipN* (CC1485)	This work
pSS125	pUT18 carrying *tipN* (CC1485)	This work
pSS135	pXCHYC-2 carrying *dipM*Δ(AA123-291)	This work
pSW15	pVCFPC-6 carrying *ftsZ*	This work
pSW61	pXCFPN-4 carrying *dipM*	This work

Table S5. Strain and plasmid construction.

Strain/Plasmid	Construction
STRAINS	
MT258	Deletion of *dipM* in CB15N by double homologous recombination using pMT814
MT251	Integration of pMT795 at the *xylX* locus of CB15N
MT261	Substitution of the native *dipM* gene of CB15N with *dipM-mCherry* by double homologous recombination using pMT816
AM128	Integration of pMT795 at the *xyl*X locus of AM52
AM192	Integration of pAM071 at the *xyl*X locus of CB15N
AM199	Integration of pAM076 at the *xyl*X locus of CB15N
AM200	Integration of pAM077 at the *xylX* locus of CB15N
AM205	Integration of pMT795 at the *xylX* locus of MT258
AM206	Integration of pSW15 at the *vanA* locus of MT251
AM214	Integration of pAM085 at the *vanA* locus of YB1585
AM216	Integration of pAM087 at the *xylX* locus of MT261
AM221	Integration of pAM078 at the *xylX* locus of CB15N
AM222	Integration of pAM078 at the *xylX* locus of MT258
AM225	Integration of pAM071 at the *xylX* locus of MT258
AM231	Integration of pAM076 at the *xylX* locus of MT258
AM232	Integration of pAM077 at the *xylX* locus of MT258
AM233	Integration of pAM079 at the *xylX* locus of CB15N
AM234	Integration of pAM079 at the *xylX* locus of MT258
AM239	Integration of pAM081 at the *xylX* locus of CB15N
AM240	Integration of pAM082 at the *xylX* locus of CB15N
AM241	Integration of pAM081 at the *xylX* locus of MT258
AM242	Integration of pAM082 at the *xylX* locus of MT258
AM263	Integration of pSPX47 at the *xylX* locus of MT258
AM264	Integration of pGB113 at the *vanA* locus of MT258
AM285	Integration of pAM100 at the *xylX* locus of CB15N
AM296	Transduction of MT258 with ΦCr30 lysate of LS4032 and transformation of the resulting strain with pEJ216
AM337	Deletion of CC1876 in CB15N by double homologous recombination using pAM123
AM342	Integration of pAM141 at the *xylX* locus of CS606
SS187	Integration of pSS135 at the *xylX* locus of MT258
SW59	Integration of pSW61 at the *xylX* locus of MT258
PLASMIDS	
pAM071	*dipM*(nt 1-1500) was PCR-amplified from CB15N chrom. DNA using primers *dipM*_uni_119 and -123r, cut with NdeI and EcoRI and ligated into equally treated pXCHYC-2.
pAM072	*dipM*(nt 79-1827) was PCR-amplified from CB15N chrom. DNA using primers *dipM*_124f and -rev2_121, cut with NcoI and EcoRI and ligated into equally treated pET28a(+).
pAM076	*dipM*(nt 1-873) was PCR-amplified from CB15N chrom. DNA using primers *dipM*_uni_119 and -128r cut with NdeI and EcoRI and ligated into equally treated pXCHYC-2.
pAM077	*dipM*(nt 1-366) was PCR-amplified from CB15N chrom. DNA using primers *dipM*_uni_119 and -129r, cut with NdeI and EcoRI and ligated into equally treated pXCHYC-2.
pAM078	Reverse PCR was performed on pMT795 using primers CC1996_132f and -133r. The product was cut with KpnI and re-ligated.
pAM079	Reverse PCR was performed on pMT795 using primers CC1996_134f and -133r. The product was cut with KpnI and self-ligated.
pAM081	Reverse PCR was performed on pMT795 using primers CC1996_137f and -133r. The product was cut with KpnI and self-ligated.
pAM082	Reverse PCR was performed on pMT795 using primers CC1996_138f and -133r. The product was cut with KpnI and self-ligated.
pAM085	*dipM* was PCR-amplified from CB15N chrom. DNA using primers CC1996-uni/-rev, cut with NdeI and EcoRI and ligated into pVCHYC-1.
pAM087	*ftsK* was PCR-amplified from CB15N chrom. DNA using primers FtsK-56f/57r, cut with NdeI and EcoRI and ligated into pXCFPC-2.
pAM091	*dipM*(nt 1501-1827) was PCR-amplified from CB15N chrom. DNA using primers CC1996_166f and -rev2_121, cut with NcoI and EcoRI and ligated into pET28a(+) cut with NcoI and EcoRI.
pAM092	*dipM*(nt 79-1500) was PCR-amplified from CB15N chrom. DNA using primers CC1996_124f and -123r, cut with NcoI and EcoRI and ligated into pET28a(+) cut with NcoI and EcoRI
pAM100	*dipM* was PCR-amplified from CB15N chrom. DNA using primers CC1996-uni/-2, cut with NdeI and EcoRI and ligated into pSTRC-2 cut with NdeI and EcoRI.
pAM102	'MAS'-CC1876(AA35-396) was PCR-amplified from CB15N chrom. DNA using primers CC1876_181f/182r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_U18_183f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pUT18C cut with PstI and EcoRI.
pAM103	CC1876(AA 35-396) was PCR-amplified from CB15N chrom. DNA using primers CC1876_181f/182r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_K25_185f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pKT25 cut with PstI and EcoRI.
pAM104	*dipM*(nt 79-1500) was PCR-amplified from CB15N chrom. DNA using primers CC1996_124f /–120r and cut with NcoI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_U18_183f and MalG_184r and with PstI and NcoI. Both fragments were ligated into pUT18C cut with PstI and EcoRI.
pAM105	*dipM*(nt 79-1500) was PCR-amplified from CB15N chrom. DNA using primers CC1996_124f/ -120r and cut with NcoI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_K25_185f and MalG_184r and cut with PstI and NcoI. Both fragments were ligated into pKT25 cut with PstI and EcoRI.

Table S5. continued

Strain/Plasmid	Construction
pAM_T25pp	*malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_K25_185f and MalG_186r, cut with PstI and EcoRI and ligated into pKT25 cut with PstI and EcoRI.
pAM_T18pp	*malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_U18_183f and MalG_186r, cut with PstI and EcoRI and ligated into pUT18C cut with PstI and EcoRI.
pAM113	CC3232 was PCR-amplified from CB15N chrom. DNA using primers CC3232-38f/46r, cut with NdeI and EcoRI and ligated into pAM111 cut with NdeI and EcoRI.
pAM114	CC3232 was PCR-amplified from CB15N chrom. DNA using primers CC3232-38f/46r, cut with NdeI and EcoRI and ligated into pAM112 cut with NdeI and EcoRI.
pAM121	'MAS'-CC1996(AA501-609) was PCR-amplified from CB15N chrom. DNA using primers CC1996_197f/120r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_K25_185f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pKT25 cut with PstI and EcoRI.
pAM122	'MAS'-CC1996(AA501-609) was PCR-amplified from CB15N chrom. DNA using primers CC1996_197f/120r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_U18_183f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pUT18C cut with PstI and EcoRI.
pAM123	The upstream and downstream regions of CC1876 were PCR-amplified from CB15N chrom. DNA using primers CC1876-203f/-204r and CC1876-205f/-206r. The reaction products were treated with HindIII/EcoRI and NheI/EcoRI, respectively, and ligated into HindIII/NheI cut pNTPS138.
pAM125	'MAS'-CC1996(AA501-609) was PCR-amplified from CB15N chrom. DNA using primers CC1996_207f/198r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_K25_185f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pKT25 cut with PstI and EcoRI.
pAM126	'MAS'-CC1996(AA501-609) was PCR-amplified from CB15N chrom. DNA using primers CC1996_207f/198r and cut with NdeI and EcoRI. *malG*(nt 1-231) was PCR-amplified from *E. coli* chrom. DNA using primers MalG_U18_183f and MalG_184r and cut with PstI and NdeI. Both fragments were ligated into pUT18C cut with PstI and EcoRI.
pAM141	*dipM* was PCR-amplified from CB15N chrom. DNA using primers CC1996-uni/-rev. The product was cut with NdeI and EcoRI and ligated into equally treated pXBlaMC-2.
pAM142	*malE* was PCR-amplified from pMal-c2e using primers MalE-233f/-234r. The product was cut with NcoI and EcoRI and ligated into equally treated pET28a(+).
pMT795	*dipM* was PCR-amplified from CB15N chrom. DNA using primers CC1996-uni/-rev. The product was cut with NdeI and EcoRI and ligated into equally treated pXCHYC-2.
pMT808	*dipM* was PCR-amplified from CB15N chrom. DNA using oligos CC1996-uni/-rev2. The product was cut with NdeI and SacI and ligated into pBXMCS-2.
pMT814	The upstream and downstream region of *dipM* were PCR-amplified from cosmid strain 1H12 using primers CC1996-1/-2 and CC1996-3/-4. The reaction products were treated with HindIII/NheI and NheI/EcoRI, respectively, and ligated into HindIII/EcoRI cut pNTPS138.
pMT816	The *dipM* downstream region was PCR-amplified from CB15N chrom. DNA using primers CC1996-3b/-4, cut with BsrGI, then treated with PNK. '*dipM-mCherry* was isolated from pMT795 by restriction with SalI, T4 pol treatment, and subsequent restriction with BsrGI. The resulting fragments were triple-ligated into pNPTS138 cut with EcoRV.
pSS85	*dipM-mCherry* was isolated from pMT795 by restriction with NdeI and NheI and ligated into pBAD24-CB cut with NdeI and XbaI.
pSS96	CC2007 was PCR-amplified from CB15N chrom. DNA using primers SS196 and SS208. The product was digested with BglII and EcoRI and ligated into pUT18C cut with BamHI and EcoRI.
pSS102	CC2007 was PCR-amplified from CB15N chrom. DNA using primers SS196 and SS208. The product was digested with BglII and EcoRI and ligated into pKT25 cut with BamHI and EcoRI.
pSS124	CC1485 was PCR-amplified using primers SS239 and SS240. The PCR product was cut with HindIII and EcoRI and ligated into equally treated pKNT25.
pSS125	CC1485 was PCR-amplified using primers SS239 and SS240. The PCR product was cut with HindIII and EcoRI and ligated into equally treated pUT18.
pSS135	Reverse PCR was performed on pMT795 using primers CC1996_fw and -133r. The product was cut with KpnI and self-ligated.
pSW15	*ftsZ* was isolated from pMT217 by restriction with NdeI and EcoRI and ligated into equally treated pVCFPC-6
pSW61	*dipM* was PCR-amplified from CB15N chrom. DNA using oligos CC1996_uni_119 and -120rev. The product was cut with NdeI and EcoRI and ligated into pXCFPN-4 cut with NdeI and EcoRI.

Table S6. Oligonucleotides

Restriction sites are indicated by capital letters.

Designation	Sequence
CC1546_194r	aaaTCTAGAtcatgtctggcctccctgcgg
CC1546_T18_193f	aaCTGCAGtCATatgagcgaaccgtccatcttc
CC1546_T25_192f	aaCTGCAGcaCATatgagcgaaccgtccatcttc
CC1876_181f	aaaaCATATGggacccgccgcgcccgccggcgtccag
CC1876_182r	taGAATTCctaagacttgcgaagacccgagga
CC1876_203f	tatAAGCTTgcgtctcttcggcgtgtgctgcgtc
CC1876_204r	taGAATTCgatgagacctctacgcattcgcgcc
CC1876_205f	taGAATTCtcgggtcttcgcaagtcttaggcgac
CC1876_206r	tttttGCTAGCgctgggtgatcgtggagccgccttc
CC1996-uni	tatatataCATatgaggcagttgtggacgcaagcgg
CC1996-rev	taGAGCTCcgcgcggcagcaccagcgccggatc
CC1996-rev2	taGAGCTCGGTACCtcagcgcggcagcaccagcgccgg
CC1996-1	atAAGCTTgatcgcctgcggccagaccatcagc
CC1996-2	tataGCTAGCagcgatcaccgccgcttgcgtccac
CC1996-3	tataGCTAGCaagccggtcgatccggcgctggtg
CC1996-4	taGAATTCgggcgctgagctcgcccgctatctc
CC1996-3b	taTGTACAagtaatcaggatgggcccgcttcgttgg
CC1996_123r	taGAATTCGCgcccgtgcccttcacgccaaagc
CC1996_124f	aaaCCATGGCGAGCcagtcgggtcagcgcttcacgcc
CC1996_128r	taGAATTCGCcgccacggtcacgaccgaaccgg
CC1996_129r	taGAATTCGCcacctggggcttgcccgcgacttt
CC1996_132f	aGGTACCgcgacgaccgccaaggcctatgtc
CC1996_133r	aGGTACCcacctggggcttgcccgcgacttt
CC1996_134f	aGGTACCgcgacgacgcagaaggcctattccg
CC1996_135r	aGGTACCcgccacggtcacgaccgaaccg
CC1996_137f	aGGTACCgacggctacaaggacaagggccc
CC1996_138f	aGGTACCgacggtttccgcgacaaggggc
CC1996_166f	aaaCCATGGCGAGCcagcgcaacgacggcctcaatatcc
CC1996_197f	ttttCATATGGCGAGCcagcgcaacgacggcctcaatatcc
CC1996_198r	taGAATTCtcagcccgtgcccttcacgccaaagc
CC1996_207f	ttttCATATGGCGAGCcagtcgggtcagcgcttcacgcc
CC1996_fw	aGGTACCgggccgcgtcaggtccacacggtc
CC1996_rev_120	taGAATTCtcagcgcggcagcaccagcg
CC1996_rev2_121	taGAATTCGCgcgcggcagcaccagcgcc
CC1996_seq1_139	gctccggtctccactcctgc
CC1996_uni_119	ttttCATATGaggcagttgtggacgcaagcggc
CC3232_38f	ttttCATatggcgatgtcctccaacgacgc
CC3232_46r	ttGAATTCgactgcgcaggccgcaggtcggccgc
MalE_233f	aaaCCATGGCGAGCatgaaaatcgaagaaggtaaactgg
MalE_234r	atGAATTCgccggtaccttgtcatcgtcatccc
MalG_184r	CCATGGtCATATGcggtggcgtaatgcgaccatcagc
MalG_186r	taGAATTCttacggtggcgtaatgcgaccatcagc
MalG_K25_185f	aaCTGCAGcaggtaccgcaatggcaatggtccaaccgaaatcg
MalG_U18_183f	aaCTGCAGtggtaccgcaatggcaatggtccaaccgaaatcg

SUPPLEMENTAL FIGURES

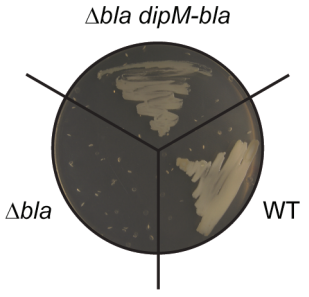

Figure S1. Subcellular localization of DipM. *C. crescentus* strains CB15N (WT), CS606 (Δ*bla*) and AM342 (Δ*bla* P*xyl*::P*xyl-dipM-bla*) were plated on PYE agar containing 50 µg/ml ampicillin and 0.3% xylose and incubated for two days at 28°C.

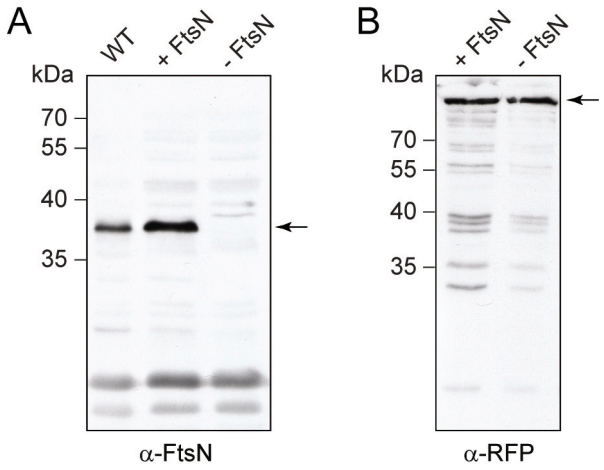

Figure S2. Stability of DipM-mCherry upon depletion of FtsN. *C. crescentus* strain AM128 (P*xyl*::P*xyl-dipM-mCherry* Δ*ftsN* P*van*::P*van-ftsN*) was grown to exponential phase in PYE medium containing 0.5 mM vanillate (+ FtsN). The cells were washed, depleted of FtsN by cultivation for another 14 h in the absence of inducer (- FtsN). Two hours before analysis, expression of *dipM-mCherry* was induced by addition of 0.3% xylose. **(A)** FtsN was detected by immunoblot analysis using anti-FtsN antiserum. For comparison, a lysate of wild-type strain CB15N (WT), grown to exponential phase in PYE medium, was included in the analysis. **(B)** DipM-mCherry was detected using anti-mRFP1 antiserum. Arrows indicate the proteins of interest.

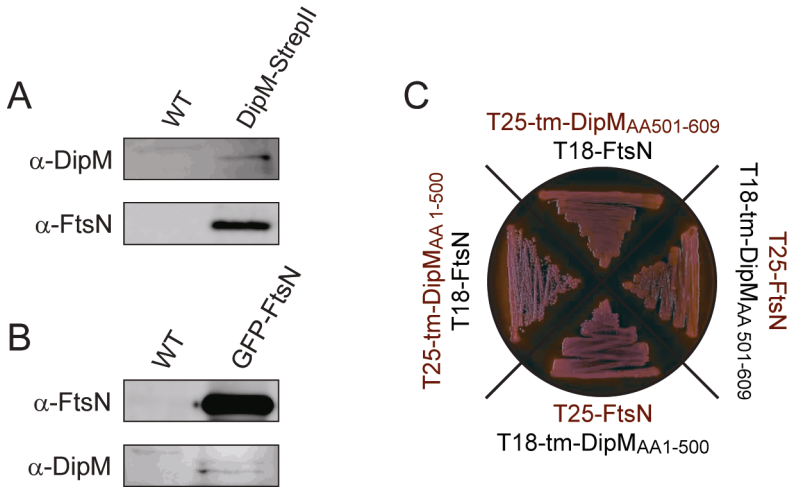

Figure S3. Interaction between FtsN and DipM. **(A)** Co-immunoprecipitation of FtsN with DipM. Strain AM285 (*dipM*::*dipM-strepII*) and wild-type strain CB15N were grown to exponential phase in M2G medium. Protein complexes were cross-linked with formaldehyde and precipitated with Strep-Tactin magnetic beads (Qiagen). The eluate of the beads was analyzed by immunoblotting using anti-DipM and anti-FtsN antibodies. **(B)** Co-immunoprecipitation of DipM with FtsN. Strain MT46 (*egfp-ftsN*) and wild-type strain CB15N were grown to exponential phase in M2G medium. After cross-linking with formaldehyde, protein complexes were immunoprecipitated with anti-GFP antibody coupled agarose (MBL). The proteins isolated were detected by immunoblotting using anti-DipM and anti-FtsN antibodies. **(C)** BACTH analysis of the interaction between FtsN and DipM. Reporter strain BTH101 was transformed with plasmids encoding fusions of *Bordetella pertussis* adenylate cyclase fragments T18 and T25 to DipM or fragments thereof. Transformants were plated on MacConkey agar supplemented with maltose. Interaction is indicated by the red coloration of the colonies.

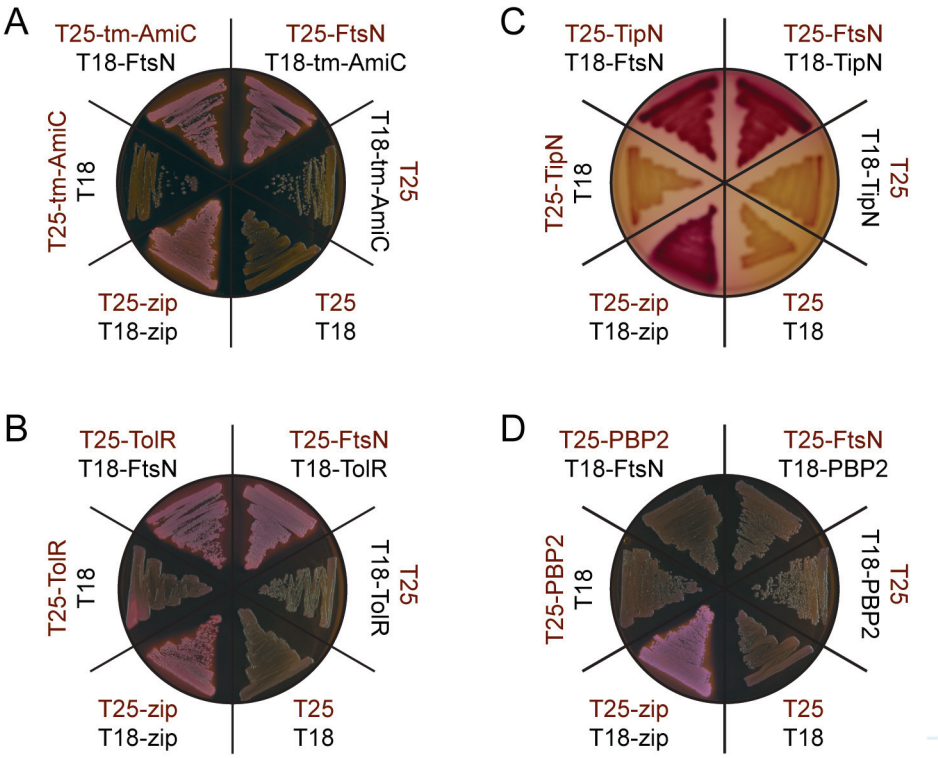

Figure S4. BACTH analysis of the interaction between FtsN and AmiC, TolR, TipN and PBP2. Reporter strain BTH101 was transformed with plasmids encoding fusions of *B. pertussis* adenylate cyclase fragments T18 and T25 to the yeast GCN4 leucin-zipper region (zip), the transmembrane linker $MalG_{AA1-77}$ (tm), $MalG_{AA1-77}$-$AmiC_{AA35-396}$ (tm-AmiC), FtsN, TipN, or PBP2. Transformants were plated on MacConkey agar supplemented with maltose. Interaction is indicated by the formation of red colonies.

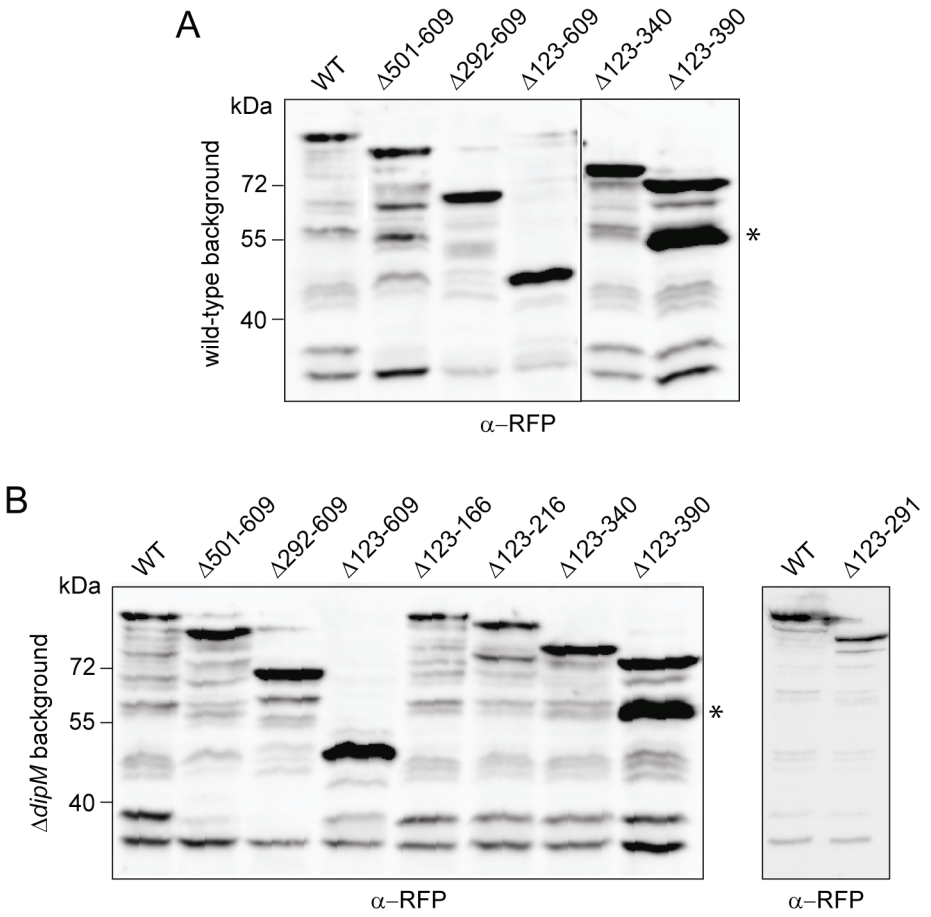

Figure S5. Stability of mutant DipM-mCherry derivatives in the wild-type and Δ*dipM* background. **(A)** Detection of DipM-mCherry derivatives in the wild-type background. Strains MT251 (DipM-mCherry, WT), AM192 (DipMΔ501-609-mCherry), AM199 (DipMΔ292-609-mCherry), AM200 (DipMΔ123-609-mCherry), AM233 (DipMΔ123-340-mCherry) and AM240 (DipMΔ123-390-mCherry) were grown to exponential phase in PYE medium, induced for 2 h with 0.3% xylose, and subjected to immunoblot analysis using anti-mRFP1 antiserum. **(B)** Detection of DipM-mCherry derivatives in the Δ*dipM* mutant background. The *dipM*-deficient strains AM205 (DipM-mCherry, WT), AM225 (DipMΔ501-609-mCherry), AM231 (DipMΔ292-609-mCherry), AM232 (DipMΔ123-609-mCherry), AM241 (DipMΔ123-216-mCherry), AM222 (DipMΔ123-166-mCherry), AM234 (DipMΔ123-340-mCherry), AM242 (DipMΔ123-390-mCherry) and SS187 (DipMΔ123-291-mCherry) were grown to exponential phase in PYE medium supplemented with 0.3% xylose and analyzed by immunoblotting using anti-mRFP1 antiserum. Stars indicate an unidentified, stable degradation product.

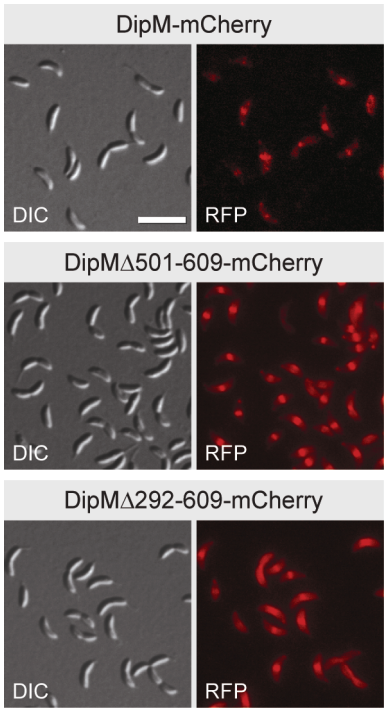

Figure S6. Localization of C-terminally truncated DipM derivatives in the wild-type background. Cells of strains MT251 (P*xyl*::P*xyl-dipM-mCherry*), AM192 (P*xyl*::P*xyl-dipM*$_{\Delta AA501-609}$*-mCherry*) and AM199 (P*xyl*::P*xyl-dipM*$_{\Delta AA292-609}$*-mCherry*) were grown in PYE medium, induced for 2 h with 0.3% xylose, and analyzed by DIC and fluorescence microscopy (bar: 5 µm).

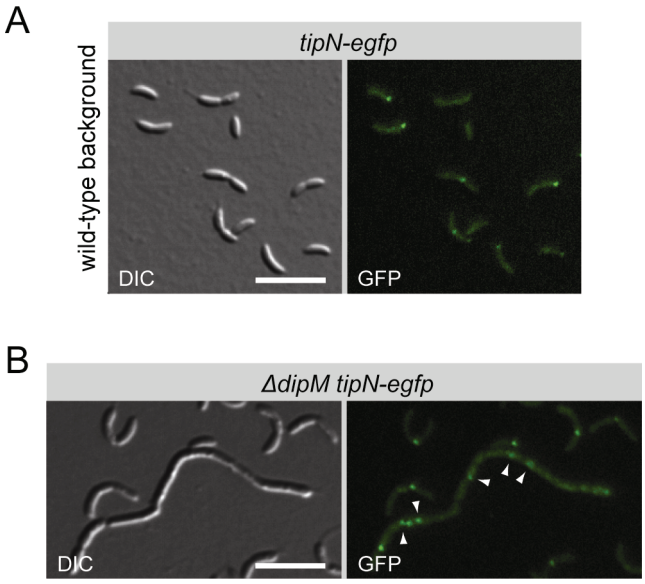

Figure S7. Localization of TipN in the wild-type and Δ*dipM* mutant background. Strains (A) NR1371 (*tipN*::*tipN-egfp*) and (B) AM263 (Δ*dipM tipN*::*tipN-egfp*) were grown in PYE medium and analyzed by DIC and fluorescence microscopy (bars: 5 µm). Arrowheads point to TipN complexes with aberrant subcellular localization.

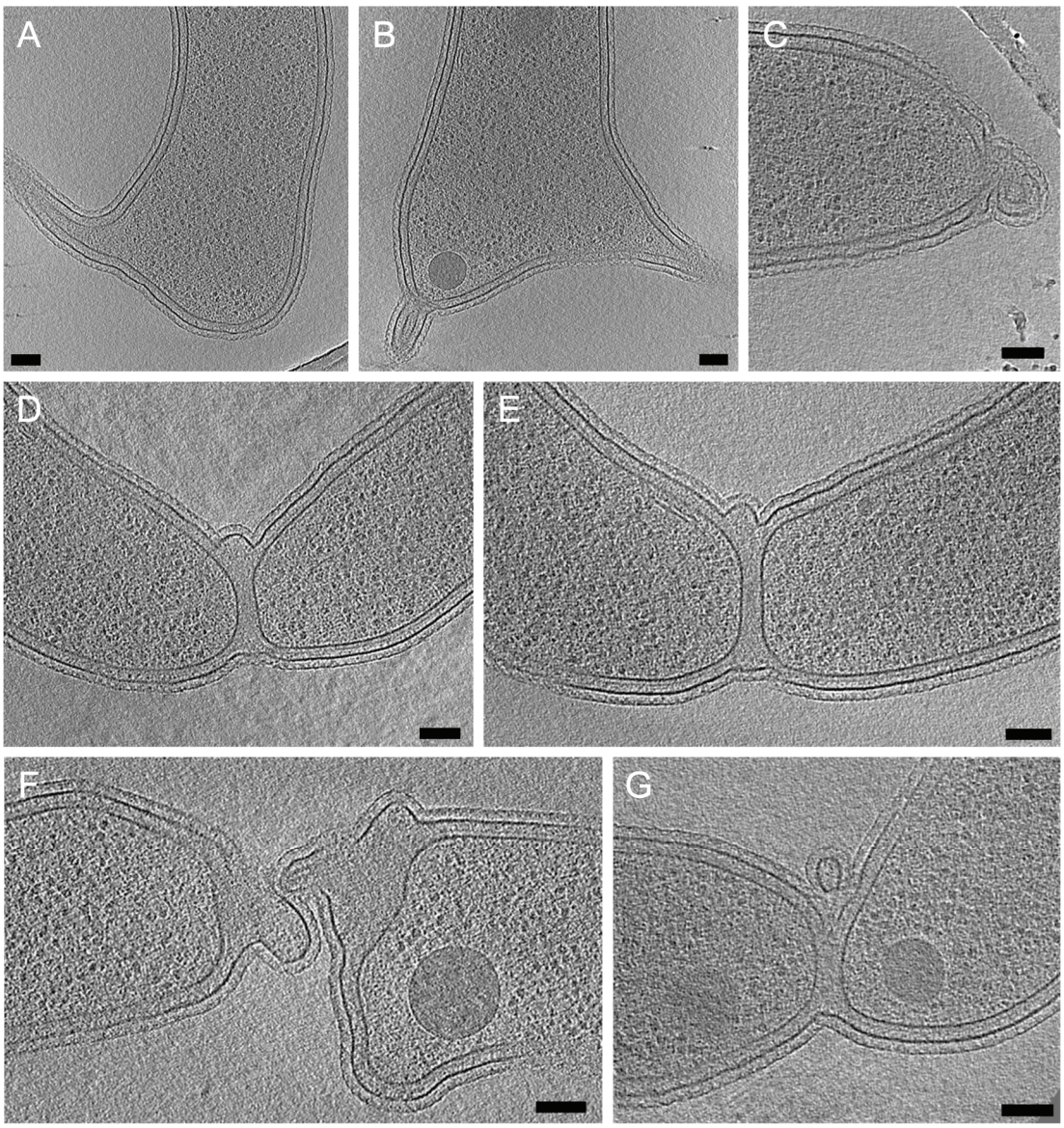

Figure S8. Cell division and polarity defects in DipM-deficient cells. Cells of strain MT258 (Δ*dipM*) were grown to exponential phase in M2G medium and analyzed by electron cryo-tomography. The images show 19-nm slices through reconstructed cells (bars: 100 nm).

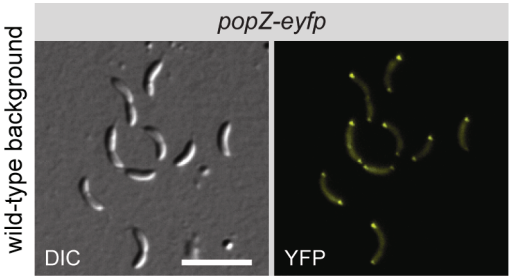

Figure S9. Localization of PopZ in the wild-type background. Strain GB175 (P*van*::P*van-popZ-eyfp*) was grown in PYE medium, induced for 1 h with 0.5 mM vanillate, and visualized by DIC and fluorescence microscopy (bar: 5 μm).

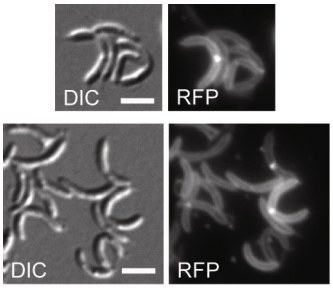

Figure S10. Enlarged periplasm at the division sites of cells lacking DipM. Cells of strain MT258 transformed with pEJ216 (P*xyl*-*torA*$_{ss}$-*tdimer2*) were grown to exponential phase in PYE medium, induced for 2 h with 0.3% xylose, and visualized by DIC and fluorescence microscopy (bar: 2.5 µm).

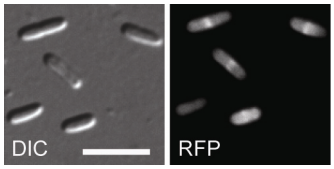

Figure S11. Recruitment of *C. crescentus* DipM to the division site of *E. coli*. Cells of *E. coli* TOP10 transformed with plasmid pSS85 (P_{BAD}-*dipM-mCherry*) were grown to exponential phase in M9-CA medium (Möll & Thanbichler, 2009) containing 0.2% glucose, washed, and resuspended in M9-CA medium supplemented with 0.2% L-arabinose. After 30 min incubation, the suspension was adjusted to 0.2% glucose and incubated for another 90 min. Subsequently, the cells were visualized by DIC and fluorescence microscopy (bar: 5 µm).

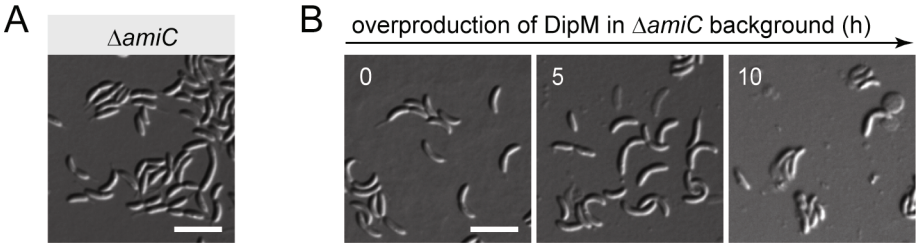

Figure S12. Role of AmiC in cell division. (A) Phenotype of an *amiC*-deficient strain. Cells of strain AM337 (Δ*amiC*) were grown in PYE medium and visualized by DIC microscopy (bar: 5 µm). **(B)** Overproduction of DipM in a Δ*amiC* background. Strain AM337 transformed with plasmid pMT808 (P*xyl*-*dipM*) was grown in PYE medium, and expression of *dipM* was induced by addition of 0.3% xylose. At the indicated timepoints, cells were withdrawn from the culture and visualized by DIC microscopy (bar: 5 µm). The culture was maintained in exponential phase throughout the course of the experiment.

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